Disk Diffusion and Serial Dilution Tests of Susceptibility of Some Pathogenic Gram-Negative Bacilli and Enterococci to Carbenicillin and Ampicillin

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Tests for susceptibility to ampicillin and carbenicillin were performed with 35 strains each of Klebsiella, Enterobacter, Serratia, and Proteus, 71 strains of Pseudomonas aeruginosa, and 68 strains of enterococci by serial dilution and disk-diffusion tests employing \(10^{-4}\) dilutions of overnight cultures as inocula for both. Commercial 10-\(\mu g\) ampicillin and 50- and 100-\(\mu g\) carbenicillin disks, and freshly prepared 10-, 50-, and 75-\(\mu g\) ampicillin and 10- and 50-\(\mu g\) carbenicillin disks were used. Results were displayed as cumulative distribution curves for both minimal inhibitory concentrations and zone diameters, and as scattergrams for correlating them. Differences in susceptibility to the two antibiotics were small for Klebsiella, Enterobacter, and Serratia and large for the others. The freshly prepared and commercial disks of the same content gave comparable zones. There was good correlation of zone diameter with each disk and the minimal inhibitory concentration. Among the ampicillin disks tested, none was useful for Pseudomonas; with the other species, the 10-\(\mu g\) disk, as well as those with higher ampicillin content, could discriminate susceptible from resistant strains. However, only the 75-\(\mu g\) disk selected some Klebsiella strains susceptible to high concentrations. The 50- and 100-\(\mu g\) carbenicillin disks were equally discriminating for most strains, but the higher concentration was more selective for Klebsiella. The 10-\(\mu g\) carbenicillin disk was as effective as the 50- and 100-\(\mu g\) disks for discriminating among Enterobacter, Serratia, Pseudomonas, and Proteus, but not for Klebsiella or enterococci. The \(10^{-4}\) inoculum gave zone sizes considerably larger than those reported by other workers who used the standard Kirby-Bauer method.

In a recent paper, Matsen et al. (12) presented a detailed comparison and evaluation of the 50- and 100-\(\mu g\) carbenicillin disks in diffusion susceptibility testing, particularly of Pseudomonas aeruginosa, but also of a wide variety of other bacterial strains: they also referred to most of the earlier relevant reports of other workers. We had been carrying out similar studies with some variations in the methods and had included simultaneous tests with disks, freshly prepared in our laboratory, containing 10, 50, and 75 \(\mu g\) of ampicillin and 10 and 50 \(\mu g\) of carbenicillin in addition to commercial 10-\(\mu g\) ampicillin and 50- and 100-\(\mu g\) carbenicillin disks. The 10-\(\mu g\) carbenicillin disk was used to explore its possible usefulness in selecting the more sensitive organisms that might respond to oral doses of indanyl carbenicillin which are recommended for the treatment of urinary tract infections, i.e., doses considerably smaller than those of intravenous carbenicillin generally employed (3, 6, 8, 13, 18, 20). The 50- and 75-\(\mu g\) ampicillin disks were tested for usefulness in selecting bacteria susceptible to concentrations of ampicillin that might be achievable in the blood, at least for short periods, with larger intravenous doses than those generally employed, or that are readily maintained or exceeded in the urine but not in the blood with smaller conventional doses and hence might also be useful in selecting this agent for therapy of urinary tract infections (10, 13, 20). Strains of Klebsiella, Enterobacter, Serratia (referred to collectively as K-E-S), Pseudomonas, Proteus, and enterococci were utilized for this study.

MATERIALS AND METHODS

The organisms used in the present study were isolated and identified from clinical specimens submitted to the Laboratory of Medical Microbiology of Boston City Hospital, about one-half of them in 1972 and the rest in late 1973 and early 1974. The cultures
were grown and identified from single colonies and maintained on heart infusion agar slants at room temperature. At the time of the tests, they were subcultured and their purity and identity were confirmed before use.

Carbenicillin disodium and ampicillin trihydrate for the serial dilution tests and for preparing our disks (identified here as G), and some commercial disks prepared by Pfizer and Baltimore Biological Laboratories (identified here as P and B, respectively), were generously supplied by Pfizer, Inc., through the courtesy of C. Douglas Webb. The G disks were prepared by placing 20 μl of the appropriate concentrations of aqueous solutions of antibiotic on paper disks (no. 740E, Schleicher & Schuell, Keene, N.H.) with a micropipette. Except when otherwise noted, aqueous solutions were freshly prepared from the stock powder for all the tests.

The plates were inoculated with cotton swabs dipped into the diluted culture, wrung out against the side of the tube, and streaked onto the full surface of the plate in three directions. The plates were allowed to dry while the disks were being inoculated. The only prediffusion took place between the time the disks were placed and the plates were put into an incubator.

The commercial disks were of recent manufacture and had been kept in sealed dessicators in a cold room until used. They were not specifically tested for potency; the similar zone diameters achieved with freshly prepared and commercial disks of the same content, and the linear regression of zone sizes and different disk content of the same antibiotic, both fresh and commercial, were considered adequate controls for the limited purposes of the present study.

Minimal inhibiting concentrations (MICs) were determined with the replica inoculating apparatus of Steers et al. (16) on Mueller-Hinton agar (Difco), pH 7.2, containing twofold dilutions of the antibiotics. The inoculum for these tests as well as for the lawn in the disk-diffusion tests, also done on Mueller-Hinton agar, was a 1:1000 dilution of an overnight (18 to 20 h) culture grown in brain heart infusion broth (Difco), pH 7.2, at 37 C; this provided a uniformly confluent growth for the lawn and the delivery of about 2,000 colony-forming units by the inoculator. This inoculum, applied on the surface of the agar plates in the manner specified in the standard Kirby-Bauer method (6), was selected for the disk diffusion tests. In previous studies with trimethoprim alone and combined with sulfamethoxazole, in which parallel tests were done employing the 10⁻³ dilutions of overnight cultures and inocula prepared by comparison of turbidity with that of the specified standard suspension, the former regularly yielded larger clear zones. In those studies, the mean and median differences for all strains of many species tested, including those used in the present studies, was 3 mm (2). In preliminary tests with several susceptible strains, this was also demonstrated with all of the disks used in the present studies. Since the 10⁻³ dilution was also used as the inoculum for determining MICs, it was simpler and more expedient to use the same dilution also for the disk diffusion tests. The tests were read after incubation overnight at 37 C. The end point for MICs was the highest concentration of antibiotic on which there was no visible growth or ≤5 small colonies visible with the aid of a 3x magnifying glass. Diameters of clear zones were read to the nearest 0.5 mm. A strain of Escherichia coli (ATCC 25922) was included in each run; the MICs of this strain in different tests varied within a single twofold dilution, and the zones with each disk generally ranged within ±2 mm.

The strains studied included 35 each of K. pneumoniae, Enterobacter, S. marcescens, and Proteus, 71 strains of P. aeruginosa, and 67 strains of enterococci. The biochemical tests used to differentiate these organisms were similar to those used in previous studies in this laboratory for Klebsiella, Enterobacter, and Serratia by Eickoff et al. (7), for Proteus by Adler et al. (1), and for enterococci by Toala et al. (17). The statistical tests used in this report were the paired t test and Nawanet linear regression analysis which was treated in detail by Snedecor and Cochran (15). The regression lines were all computed as regressing log₁₀ MIC on zone diameters.

RESULTS

Although the results obtained with the different species showed some similarities, they differed quantitatively and in relative susceptibility to the two penicillins. For that reason and for clarity and convenience, the results will be presented separately for the strains of each species.

K. pneumoniae. The MICs of ampicillin and carbenicillin for the 35 strains of K. pneumoniae are shown as cumulative distribution curves in Fig. 1. The distribution for each antibiotic is bimodal, with 60% of the strains inhibited by 6.3 to 50 μg of ampicillin per ml and by an average of four times these concentrations of carbenicillin. The differences in MIC of individual strains ranged from one-half (1 strain) to eightfold (three strains).

![Fig. 1. Cumulative distribution curves for susceptibility of 35 strains of Klebsiella pneumoniae to ampicillin and carbenicillin.](http://aac.asm.org/Downloaded from http://aac.asm.org/ on June 16, 2017 by guest)
The cumulative distribution curves of zone diameters produced by the ampicillin and carbenicillin disks are shown in Fig. 2. It should be noted that the abscissa shows increasing zone diameters, corresponding to increasing susceptibility from left to right. This is in contrast to the abscissa in Fig. 1 which shows increasing concentration of antibiotic, corresponding to decreasing susceptibility, from left to right. The curves for both 10-μg ampicillin disks were very similar \((P = 0.32)\). The zones produced by the 50-μg disks were, on the average, \(5.3 \pm 0.2\) mm larger than those of the 10-μg disks \((P < 0.001)\), and the difference in mean diameters of the zones obtained with the 50- and 75-μg disks was \(1.5 \pm 0.1\) mm \((P < 0.001)\).

The commercial 50-μg carbenicillin disk \((B, 50)\) generally produced larger zones than the freshly prepared one \((G, 50)\) by an average of 0.9 \(±\) 0.2 mm \((P < 0.01)\). The difference between the zone diameters produced by each 50-μg disk and the 100-μg disk was highly significant \((P < 0.001)\). The paired \(t\) test was used for comparing zone diameters.

Levels of 2 and 4 μg of ampicillin per ml are usually achieved in serum with oral doses of 500 and 1,000 mg, respectively. Concentrations of carbenicillin in serum usually reach 10 to 12 μg/ml after doses of 1.0 g of indanyl carbenicillin \((11)\). None of the strains of Klebsiella tested were inhibited by less than 6.3 μg of ampicillin or (with one exception) by less than 25 μg of carbenicillin/ml; hence, neither of these agents can be considered useful for therapy of Klebsiella infections except for bacteriuria, since concentrations of 200 μg of ampicillin and more than 1,000 μg of carbenicillin can usually be sustained in the urine of individuals receiving those doses four times daily.

Correlations of zone diameters and MICs are shown in Fig. 3. There appears to be no advantage of the 50- or 75-μg ampicillin disks over that of 10-μg content except for the larger zone diameters they produced. The 75-μg disk also gave clear zones with some of the strains, with MIC ≥ 400 μg/ml. The 10-μg carbenicillin disk produced no zones (or zones of <11 mm) with a large proportion of strains that had produced clear zones of 13 mm or greater when the 50- and 100-μg disks were used. Thus, the 50- and 100-μg disks but not the 10-μg disk are potentially useful in selecting strains of Klebsiella that might be suitable for therapy of bacteriuria with carbenicillin. The 100-μg disk but not the 50-μg disk also produced small zones with three of the more resistant strains.

**Enterobacter.** Results of the serial dilution tests for susceptibility of the 35 strains of Enterobacter are shown in Fig. 4. Ampicillin was appreciably more active than carbenicillin against a great majority of the strains. The distribution of all strains is bimodal; however, this is due to the inclusion of the strains of E. cloacae, six and eight of which were much less susceptible than the others to ampicillin and carbenicillin, respectively. The Mann-Whitney \(U\) test showed statistically significant differences between the two species for both ampicillin \((P < 0.01)\) and carbenicillin \((P < 0.005)\).

The results of the disk diffusion tests are shown in Fig. 5 as cumulative distribution curves of zone diameters for each of the ampicillin and carbenicillin disks. The plates with seven strains of E. cloacae were unsatisfactory.
The zones produced by ampicillin were all sharply defined, whereas the borders of those produced by carbenicillin were somewhat fuzzy, and only the diameters of the clear portions were recorded. The bimodal distribution of zone sizes with carbenicillin resulting from the similar distribution of MICs with both species is evident; the curves for the ampicillin disks suggest a trimodal distribution, reflecting the different distributions of the strains of the two species.

The commercial 10-μg ampicillin disk (P, 10) produced slightly but not significantly larger zones than our 10-μg disk (G, 10). With the 50-μg ampicillin disk, the zones were larger by an average of about 5 mm. The 75-μg ampicillin disk yielded zones that were generally 2 mm larger than those produced by the 50-μg disk.

The 10-μg carbenicillin disk gave zones of 19.5 to 29 mm with all except four of the strains (all E. cloacae). With the commercial 50-μg disk, zones averaged 1.5 mm larger than those around our 50-μg disk (P < 0.01) and more than 7 mm larger than those produced by our 10-μg disk. The mean difference in zone diameter produced by the 100- and 50-μg commercial disks was 2.6 mm (P < 0.001).

Correlations of MICs and zone sizes produced by each of the disks are shown, with the regression lines, in Fig. 6. Good correlations were determined for all the disks (P < 0.001). For the strains of Enterobacter that were tested, the 50- and 75-μg ampicillin disks were no more discriminating than the standard 10-μg disk; the 50- and 100-μg carbenicillin disks and also the 10-μg carbenicillin disk were all equally discriminating.

S. marcescens. The 35 strains of S. marcescens differed from those of Klebsiella and Enterobacter in that they were more or less similar in susceptibility to ampicillin and carbenicillin; 20 of them were resistant to both (MIC ≥ 400 μg/ml) and the other 15 were inhibited by <12.5 μg/ml (Fig. 7). Cumulative distribution curves of zone diameters in the disk diffusion tests are shown in Fig. 8. The results are shown only for the two commercial carbenicillin disks, and the commercial 10-μg and our 50- and 75-μg ampicillin disks with which all 35 strains were tested.

The commercial 10-μg ampicillin disk (B, 10) produced “no zones” with 20 strains, and our 50- and 75-μg disks gave zones of ≤11 mm with only three and four of the same strains, respectively. The mean difference between zones produced by the 10- and 50-μg disks was 3.5 mm (P < 0.001); between the 50- and 75-μg disks the difference was 2.1 mm (P < 0.001).

Ten of the strains were also tested with 10-μg ampicillin and 50- and 100-μg carbenicillin disks prepared in our laboratory as controls for comparison with commercial disks of corre-
Fig. 5. Left: Cumulative distribution of diameters of zones of inhibition of 28 strains of Enterobacter by four carbenicillin disks (upper panel) and by four ampicillin disks (lower panel). Right: Similar curves showing cumulative distribution of zone sizes of 11 strains of E. aerogenes and 17 strains of E. cloacae with the carbenicillin disks (above) and ampicillin disks (below).

Fig. 6. Scattergrams showing correlation of MIC of ampicillin and zone diameters produced by four ampicillin disks with 28 strains of Enterobacter (upper) and similar correlations for MIC of carbenicillin and four carbenicillin disks with the same strains.
The resulting content; the results were identical for each strain.

No zones were produced by either the 50- or 100-µg carbenicillin disk with the same 20 strains. The average difference in zone diameters produced by the 100- and 50-µg disks with the "sensitive" strains was 2.4 mm (P < 0.001).

MICs and zone diameters for the 35 strains of Serratia are correlated in the scattergrams shown in Fig. 9. All the "resistant" strains grew to the disk margins, except three, which had an MIC of ampicillin at 400 µg/ml, and gave zones of 7 to 11 mm with the 50- and 75-µg ampicillin disks. Although the number of "sensitive" strains was small, there was good correlation between MIC and zone size with those strains for each of the five disks used, and all the disks were equally discriminating.

P. aeruginosa. Cumulative distribution curves of MICs of carbenicillin for the 71 strains of P. aeruginosa are shown in Fig. 10. They were tested in two batches: 35, which were isolated late in 1973, were tested on one day; 36, which were isolated early in 1974, were tested 3 days later. Portions of the concentrated solutions of antibiotics that were used in the first batch, and which were kept frozen at -20°C in the interim, were used for the second batch. The difference between the two tests was due to the inclusion in the second set of two strains that produce

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Fig. 7. Cumulative distribution curves of susceptibility of 35 strains of Serratia marcescens to ampicillin and carbenicillin.

Fig. 8. Cumulative distribution of diameters of zones of inhibition of 35 strains of Serratia marcescens by 50- and 100-µg carbenicillin disks (above), and by 10-, 50-, and 75-µg ampicillin disks (below).

Fig. 9. Upper panel: Scattergrams showing correlations of MIC of carbenicillin and zone sizes obtained with 35 strains of Serratia marcescens, using 50- and 100-µg carbenicillin disks. Lower panel: Similar correlations of MIC of ampicillin with diameters of zones of inhibition produced by 10-, 50-, and 75-µg ampicillin disks.
brown pigment and two others which together accounted for the four strains with MIC ≤ 6.3 μg of carbenicillin per ml. Of the strains tested, 90% were inhibited by ≤50 μg of carbenicillin per ml, and the MIC of seven strains was 100 or 200 μg of carbenicillin per ml.

The “brown” strains were also inhibited by 50 μg of ampicillin per ml and two other strains were inhibited by 400 μg/ml of that antibiotic, the highest concentration used in the tests. The remaining strains were all resistant to 400 μg of ampicillin per ml.

Diffusion tests were done with four ampicillin and four carbenicillin disks similar to those used in the tests of the strains of Klebsiella and Enterobacter. The two 10-μg ampicillin disks gave clear zones, 9 to 16.5 mm in diameter, with only five strains, namely, the two brown-pigment producers, one strain with an MIC of 400 μg/ml, and two strains that were more resistant. The freshly prepared 50-μg ampicillin disk (G, 50) produced 19- to 25-mm zones with the same five strains and 8-mm zones with two additional ampicillin-resistant strains. Our 75-μg ampicillin disks (G, 75) produced zones that were 2 to 3 mm larger with each of these seven strains, and 8- to 11-mm zones with three additional ampicillin-resistant strains. All other strains grew to the disk margins of each of the ampicillin disks. The results obtained with the ampicillin disks are not shown in the figures.

The cumulative distribution of diameters of clear zones produced by the four carbenicillin disks are shown in the upper panel of Fig. 11. Zones produced by the two 50-μg carbenicillin disks on the individual strains showed differences ranging up to 2.5 mm, more of the strains giving larger zones with the commercial (B, 50) disk than with ours (G, 50); the mean difference was 0.5 mm (P < 0.02); median zone size with these two disks was the same and was 9.5 mm larger than that of the 10-μg disk. In comparisons of the zones produced by the commercial 50- and 100-μg disks, the latter were 1 to 6 mm (average 3.2 mm) larger (P < 0.001).

The lower panel of Fig. 11 shows that the disks of each carbenicillin content discriminated about equally well among: (i) the least susceptible strains (MIC 200 μg/ml), (ii) the great majority of susceptible strains (MIC 12.5 to 50 μg/ml), and (iii) the most susceptible ones (MIC < 6.3 μg/ml). Different zone diameters delineated each of these groups for each disk content, with one or two deviant strains falling outside the set limits.

From both the cumulative distribution curves and the correlations shown in the scattergram in Fig. 11B, there appears to be no particular advantage for either the 50- or 100-μg disk, except for the larger zones produced by the latter, particularly with the least susceptible strains. The 10-μg disk appeared to discriminate best among the most and the least susceptible strains. Thus, there could be some use for the smaller disk, when therapy with the small oral doses of indanyl carbenicillin is contemplated, and when low blood levels may be expected as in therapy of urinary tract infections.

Proteus. Cumulative distribution curves for susceptibility of Proteus (Fig. 12) showed each of the penicillins to be bimodal, more than one-half of all the strains being inhibited by 0.2 to 0.8 μg of carbenicillin per ml and most of the others by concentrations up to 100 μg/ml. Ampicillin inhibited 11 strains in concentrations of 0.2 to 3.1 μg/ml, and the MIC of the other ranged from 12.5 to >400 μg/ml.

The differences in MICs among the four species of Proteus are more clearly demonstrated in Fig. 13. Although the numbers of strains are small, those of each species show a distinctive pattern of susceptibility which is different for each antibiotic; however, all showed greater susceptibility to carbenicillin than to ampicillin. The strains of P. mirabilis are the most uniformly susceptible to both penicillins and show the least differences in susceptibility to the two penicillins.

The cumulative distribution curves of zone diameters produced with Proteus by the different disks are shown in Fig. 14. For each of the penicillins, the curves for all strains exhibited a trimodal distribution which, from inspection of those of the separate species, are seen to be a
result of the latter. The two 10-μg ampicillin disks, except at the lower end of the scale, gave zones of nearly similar size, which differed from each other by an average of 0.2 mm \((P = 0.072)\). The differences in the size of zones for individual strains produced by the 10-μg ampicillin disks ranged from 0 to 6 mm.

The differences between the size of zones produced by the 10- and 50-μg ampicillin disks are obscured by the relatively larger zones produced by our 10-μg disk (G, 10) with some of the strains at the lower end of the scale. However, the 75-μg disk produced zones averaging 3.8 mm larger than those given by the 50-μg disks \((P < 0.001)\). The differences between the zones produced with the individual strains by these two disks showed much less divergence than with the 10-μg disks.

The 100-μg carbenicillin disk gave zones that averaged 2.3 mm larger than those produced by the 50-μg disks \((P < 0.001)\). Comparisons of zone diameters produced by each strain with the two 50-μg carbenicillin disks and by the 50- and 100-μg carbenicillin disks showed differences that were fairly uniform and fell within narrow ranges \((P < 0.001)\).
The marked differences in the patterns of distribution of zone sizes with both the ampicillin and carbenicillin disks with strains of the different species are clearly evident from the panels on the right in Fig. 14. The relatively large zones produced by strains of all four species by the carbenicillin disks (upper half of the figure) and the generally smaller ones produced by most strains with the ampicillin disks for all species except P. mirabilis is evident from the lower half of Fig. 14. The largest zones were produced by P. mirabilis with both penicillins. Most strains of P. mirabilis gave the next largest zones with the carbenicillin disks. A few strains of species other than P. mirabilis also gave large zones with the ampicillin disks.

A correlation of MICs and zone diameters for all 35 strains of Proteus and for all eight disks, as well as regression lines for each of the disks, are presented in Fig. 15. There was a good overall regression (P < 0.001) and reasonably good break-points in each instance. The ampicillin disks were fairly but not fully discriminatory, and even the 10-μg disk gave large clear zones, with some strains moderately resistant to ampicillin in the serial-dilution test. The 10-μg carbenicillin disk was at least as discriminating as the others, and there appeared to be no advantage of the 100-μg disk over the 50-μg disks.

Enterococci. Of the 67 strains of enterococci tested, 34 were isolated in 1972 and 33 in late 1973 or early 1974; most of them were obtained from urine in significant numbers (>10^8 colony-forming units per ml), and as the only or predominant organism. A few were isolated from sputum, blood, or infected exudates. Cumulative distribution curves of the MICs of ampicillin and carbenicillin for all 67 strains, and separate ones for those of each of the three species which they comprised, are shown in Fig. 16. The striking differences in susceptibility of all the strains to the two penicillins is at once apparent, the MICs of carbenicillin generally being 64 times those of ampicillin. Also, nearly all the strains were susceptible to each antibiotic within a fourfold range of concentration. A few of the strains of Streptococcus zymogenes were somewhat more susceptible, and some of those of S. liquefaciens slightly less so than the ones classified as S. faecalis.
The five disks used to obtain the results shown in Fig. 17 included the commercial 10-µg ampicillin and 50- and 100-µg carbenicillin disks and the 10-, 50-, and 75-µg ampicillin disks freshly prepared in this laboratory. The zones produced by the commercial 10-µg ampicillin disk and by the freshly prepared disks were either the same size or varied by <1.0 mm (not statistically significant); hence, for clarity in representing the results, only those obtained with the commercial 10-µg ampicillin disk are included in the figure. The 10-µg carbenicillin disk produced no zone or only small ones of <10 mm with all but a few strains, which are also not shown. As with the MICs, the zone diameters produced by each disk with all the strains varied within a relatively narrow range. The 50-µg carbenicillin disk (B, 50) produced the smallest zones; the 100-µg carbenicillin disk (B, 100) and the 10-µg ampicillin disk (P, 10) produced zones of very similar size (P > 0.05). The average difference in diameter between the 50- and 100-µg carbenicillin disks was 2.2 mm (P < 0.001); between the 10- and 50-µg ampicillin disks it was about 5 mm (P < 0.001), and between the 50- and 75-µg ampicillin disks the mean difference was 1.7 mm (P < 0.001). The zones produced by individual strains with the 100-µg carbenicillin disk were 1 to 4 mm larger than those with the 50-µg disk.

The cumulative distribution curves of zone diameters produced by the strains of different species are shown in the panels on the right side of Fig. 17. Some strains of *S. faecalis* produced the smallest zones, those of *S. zymogenes* gave some of the largest zones, and the strains of *S. liquefaciens* varied within a narrower range than the others.

After 1 week, a different commercial lot of 50-µg disks and the same lot of 100-µg disks were used to repeat the tests with carbenicillin. Similar variations in zone sizes obtained with the individual strains in the two sets of tests were noted with the 50- and 100-µg disks (P = 0.014). Scattergrams of zone sizes related to MICs are shown in Fig. 18 for the three commercial disks. It shows the wide range of zone diameters for each MIC and much overlap. Good correlations of MIC and zone diameter are shown for each of the three commercial disks, but there was no clear discrimination between the carbenicillin disks within the narrow range of MICs represented, and thus, the data did not permit calculation of satisfactory regressions for the individual species.
Fig. 15. Scattergrams showing correlation of MICs of ampicillin with zone diameters produced with 35 strains of four species of Proteus by four ampicillin disks (upper panel) and similar correlations of MICs of carbenicillin and zone diameters obtained with four carbenicillin disks. Regression lines are shown for each of the disks.

Fig. 16. Cumulative distribution curves of susceptibility of 67 strains of three species of enterococci to ampicillin and carbenicillin.
DISCUSSION

Infection with *P. aeruginosa* is currently the most important indication for carbenicillin, in part because it is the only nontoxic drug to which strains of this organism are susceptible in clinically achievable concentrations, and also because high levels can be sustained with relative freedom from untoward effects (9). In addition, the 5-indanyl ester of carbenicillin provides an oral form which, taken in rather small doses, yields high concentrations of active carbenicillin in the urine that have proved useful in the therapy of urinary tract infections (6).

Carbenicillin is also active against many strains of *Proteus* that are relatively resistant to ampicillin. Organisms of the *Klebsiella, Enterobacter, Serratia* group are being encountered with increasing frequency within hospitals. Ampicillin and carbenicillin are both active against these organisms, the former being more active against *Klebsiella* and *Enterobacter* and both being equally active against *Serratia*, although the majority of strains are resistant to both. Strains of enterococci are included in this study because they are being encountered frequently as a cause of residual, recurrent, and chronic infections, some of them in association with or following infections with gram-negative bacilli for which either of these penicillins may be used.

Although one purpose of the present study was to compare the usefulness of carbenicillin disks of 50- and 100-μg content, this has been done more thoroughly and effectively by other workers, particularly by Matsen et al. (12), who also reviewed previous reports, all of which utilized essentially the standard Kirby-Bauer method. They assayed and compared carbenicillin disks supplied by the same or different manufacturers and found wide variations from the labeled content; the differences were greater among 50-μg disks than among those labeled for 100-μg content, most often yielding considerably more than the labeled content. They also prepared carbenicillin disks in 10-μg increments, tested them with strains of three differ-
ent species of organisms, and demonstrated a continual linear relationship between zone diameter and disk content for each species. In addition, they showed irregular variations when different batches were used. They concluded that the 100-μg carbenicillin disk was generally more useful clinically.

Barry and Effinger (4) compared the standard Kirby-Bauer method and the agar-overlay method. They found the two methods equally satisfactory for most organisms, but the Kirby-Bauer method was more satisfactory for tests with *P. aeruginosa*, and they concurred in selecting the 100-μg carbenicillin disk over the 50-μg disk for clinical use.

In this paper, comparisons of the 50- and 100-μg carbenicillin disks were made using differences in details of method which had been found useful in previous studies with trimethoprim and sulfamethoxazole, alone and in combination (2). First, the inoculum for both the agar-dilution and disk-diffusion tests was a 10⁻³ dilution of an overnight culture. This provided an adequate but less dense lawn yielding appreciably larger zones that might provide better discrimination among organisms of different susceptibility, although no attempt was made to document this here. The performance of different disks was displayed with the use of cumulative distribution curves of zone sizes, a method used in this and most other laboratories for summarizing and comparing the results of serial dilution tests of susceptibility of organisms of different species to the same antibiotic or the activity of different antibiotics against isolates of the same species. This permitted a simple visual estimate of the variations in the performance of the same disk and more ready comparisons of the results obtained with different disks. Differences between the zone sizes produced by disks with equal amounts of ampicillin or carbenicillin were generally small, although the 50-μg commercial disk gave significantly larger zones than the freshly prepared one. This is consistent with the considerably greater than labeled content of most of the commercial disks assayed by Matsen and co-workers (12).

Ampicillin, the first of the semisynthetic penicillins that proved useful against gram-negative bacillary infections, was also used for direct comparisons with carbenicillin in both serial dilution and disk diffusion tests. For the strains of each of the species studied here, comparisons were shown of the activity of ampicillin and carbenicillin by the serial dilution test and for the distribution of zone sizes produced by each of the disks. Correlations of MICs of each of the penicillins and zone diameters produced by each of the disks were shown in scattergrams for the strains of each organism.

Direct comparisons with the standard Kirby-Bauer method were not done, but in a few preliminary tests the zone diameters demonstrated with the use of a 10⁻³ dilution for inocula were appreciably larger, confirming the previous findings with the same disks of trimethoprim alone or with sulfamethoxazole in tests of strains of the same bacterial species (2), but the discrimination between sensitive and resistant strains appeared to be comparable. Comparison of the distribution of zone sizes obtained with the standard Kirby-Bauer method of preparing the inoculum, as reported for *Pseudomonas* by Barry and Effinger (4), Matsen et al. (12), Schoenknecht (14), and Washington et al. (19), and for *Proteus* by Schoenknecht (14) indicate that the zones for strains of the corresponding species were roughly 5 to 7 mm larger, on the average, in the present study.

There was some suggestion, from the results obtained here, that an ampicillin disk of greater content than the standard 10 μg might
be useful in identifying strains which are susceptible to concentrations achievable in the urine but considered resistant by results obtained with the 10-μg disks. This may have clinical implications because of the good correlation between efficacy against urinary tract infection and concentrations contained in the urine (10).

Although a comparison of the usefulness of the 50- and 100-μg carbenicillin disks was not the primary objective of the present study, the results indicate that the 100-μg disk may have some slight advantage in selecting strains of *Klebsiella* susceptible to concentrations at the higher levels attainable in serum with large parenteral doses. However, for the strains of *Enterobacter* and *Serratia* that were tested, the 50- and 100-μg carbenicillin disks, and even the one containing 10 μg, were equally discriminating.

Almost all 71 strains of *Pseudomonas* that were tested were resistant to ampicillin (MIC > 400 μg/ml) and very few produced measurable clear zones even with a disk containing 75 μg of that antibiotic; they were all susceptible to 200 μg or less of carbenicillin, about 90% being inhibited by ≤50 μg/ml. There were good correlations of MICs and zone diameters for each of the four carbenicillin disks tested. However, the 100-μg disk was not more discriminating than the 50-μg one in selecting the least or the most susceptible strains. The freshly prepared 10-μg carbenicillin disk produced no zones with the least sensitive strains and much smaller zones than the 50- and 100-μg disks with the others, but it did distinguish the most sensitive strains and thus may be potentially useful in selecting patients for therapy with relatively small oral doses of indanyl carbenicillin.

The strains of *Proteus* were nearly all more susceptible to carbenicillin than to ampicillin and the differences were generally large for those of each species except *P. mirabilis*, which were more uniformly susceptible to both penicillins. Strains of each of the other *Proteus* species presented different and distinctive bi-modal distribution patterns of susceptibility to both antibiotics with both methods, but the numbers tested were much smaller. It would be of interest to delineate the differences more clearly with larger numbers of strains of each species. Here, as with *Klebsiella*, *Enterobacter*, and *Pseudomonas*, there might be a place for a disk of 10 μg, or at least one with less than 50 μg of carbenicillin, to select the more susceptible strains for therapy with small oral doses of indanyl carbenicillin. Likewise, disks of greater ampicillin content than the standard 10 μg—for example, 50 μg—might be useful to detect the strains considered relatively resistant for systemic infections but possibly useful for ampicillin therapy of urinary infections.

Enterococci differed from all the other species in several important ways in susceptibility patterns. Strains of enterococci were quite uniform in their susceptibility to both ampicillin and carbenicillin, each within a narrow range of MIC, and also yielded zone diameters within a narrow range. There would be no particular advantage for ampicillin disks with contents greater than the standard 10 μg. There also appears to be no demonstrable advantage for using carbenicillin for therapy of enterococcal infections, although nearly all the strains were susceptible within a range achievable with large parenteral doses, and most of them within a range achievable in the urine with the usually recommended oral doses of indanyl carbenicillin. There appears to be no advantage of the 100-μg disk over that containing 50 μg. In mixed infections of the urinary tract involving gram-negative bacilli and enterococci, carbenicillin may be a drug of choice for the former and could also serve for the latter, thus avoiding the use of ampicillin. However, from the data presented, there appears to be no good reason to test strains of enterococci for susceptibility to either carbenicillin or ampicillin except where strains resistant to these agents are known to be prevalent.

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ADDENDUM IN PROOF


LITERATURE CITED

